

Agilent Ref: 10031269-1  
United States Application Serial No. 10/712,741

### **AMENDMENTS**

#### **In the Specification:**

**Please replace paragraph 11, with the following rewritten paragraph:**

~~FIGS.~~ **FIG. 2** shows an exemplary embodiment of a degassing backing element according to the subject invention.

**Please replace paragraph 19, with the following rewritten paragraph:**

Any given substrate may carry one, two, four or more ~~or more~~ arrays disposed on a front surface of the substrate. Depending upon the use, any or all of the arrays may be the same or different from one another and each may contain multiple spots or features. A typical array may contain more than ten, more than one hundred, more than one thousand more than ten thousand features, or even more than one hundred thousand features, in an area of less than 20 cm<sup>2</sup> or even less than 10 cm<sup>2</sup>. For example, features may have widths (that is, diameter, for a round spot) in the range from ~~[[a]]~~ 10 µm to 1.0 cm. In other embodiments each feature may have a width in the range of 1.0 µm to 1.0 mm, usually 5.0 µm to 500 µm, and more usually 10 µm to 200 µm. Non-round features may have area ranges equivalent to that of circular features with the foregoing width (diameter) ranges. At least some, or all, of the features are of different compositions (for example, when any repeats of each feature composition are excluded the remaining features may account for at least 5%, 10%, or 20% of the total number of features). Interfeature areas will typically (but not essentially) be present which do not carry any polynucleotide (or other biopolymer or chemical moiety of a type of which the features are composed). Such interfeature areas typically will be present where the arrays are formed by processes involving drop deposition of reagents but may not be present when, for example, light directed synthesis fabrication processes are used. It will be appreciated though, that the interfeature areas, when present, could be of various sizes and configurations.

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**Please replace paragraph 75, with the following rewritten paragraph:**

The degassing zone may encompass an area directly opposite the one or more arrays used in the array assay and may be substantially the same ~~[[the]]~~ size, including the same size, as the area encompassing the one or more arrays to be used in the array assay. In such embodiments, the rate of elimination of bubbles over the one or more arrays will primarily be a function of the permeability of the degassing membrane. In certain embodiments, the degassing zone may be smaller than the area encompassing the one or more arrays. In such embodiments where the degassing zone area is less than that of the area of the one or more arrays, or if the degassing membrane is not directly opposite the one or more arrays, the rate of elimination of bubbles not directly opposite the one or more arrays will primarily be a function of diffusion through the liquid. In such embodiments when the primary mechanism of bubble removal is diffusion, the bubbles closest to the membrane will typically be eliminated more quickly ~~[[then]]~~ than the bubbles further away from the membrane. Agitation or mixing of the solution in the diffusion-limited case may be employed to increase the rate of bubble elimination by more quickly transporting solution with a lower concentration of dissolved gas to bubbles that are over the array surface.

**Please replace paragraph 83, with the following rewritten paragraph:**

The gas permeable membrane may be porous or non-porous, but in many embodiments is non porous and liquid impermeable. The gas permeable membrane may be permeable to one or more of a wide variety of gaseous components which exist in the gaseous state, i.e., as a gas at atmospheric pressure and about from 3°C to 86°C, including low molecular weight chemical substances that ~~[[exits]]~~ exists in the gaseous state. The gaseous component(s) may be a pure substance or a mixture and may be any of a broad range of chemical species including, but not limited to, gases such as helium, hydrogen, neon, nitrogen, argon, oxygen, ozone, carbon dioxide, ~~ozone~~, and any mixture of any of them. The subject gas permeable

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membranes may be suited for evacuation of oxygen and oxygen mixtures such as oxygen/ozone, oxygen/nitrogen, oxygen/nitrogen/ozone, and the like.

**Please replace paragraph 106, with the following rewritten paragraph:**

Any given substrate may carry one, two, four or more ~~or more~~ arrays disposed on a front surface of the substrate. Depending upon the use, any or all of the arrays may be the same or different from one another and each may contain multiple spots or features. A typical array may contain more than ten, more than one hundred, more than one thousand more than ten thousand features, or even more than one hundred thousand features, in an area of less than 20 cm<sup>2</sup> or even less than 10 cm<sup>2</sup>. For example, features may have widths (that is, diameter, for a round spot) in the range from a 10 μm to 1.0 cm. In other embodiments each feature may have a width in the range of 1.0 μm to 1.0 mm, usually 5.0 μm to 500 μm, and more usually 10 μm to 200 μm. Non-round features may have area ranges equivalent to that of circular features with the foregoing width (diameter) ranges. At least some, or all, of the features are of different compositions (for example, when any repeats of each feature composition are excluded the remaining features may account for at least 5%, 10%, or 20% of the total number of features). Interfeature areas will typically (but not essentially) be present which do not carry any polynucleotide (or other biopolymer or chemical moiety of a type of which the features are composed). Such interfeature areas typically will be present where the arrays are formed by processes involving drop deposition of reagents but may not be present when, for example, light directed synthesis fabrication processes are used. It will be appreciated though, that the interfeature areas, when present, could be of various sizes and configurations.

**Please replace paragraph 116, with the following rewritten paragraph:**

Embodiments may include samples that include a hybridization solution. In practicing the subject methods, a hybridization solution (or other analogous array assay solution) may first be prepared or may be purchased already prepared and

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ready for use in a hybridization assay. A variety of different hybridization solutions are known in the art and may be employed with the subject invention such as the hybridization solution described in U.S. Patent No. 6,258,592, the disclosure of which is herein incorporated by reference. Other hybridization solutions that may be employed in the subject invention are known to those of skill in the art and include, but are not limited to, e.g., hybridization solutions provided by Agilent Technologies, Inc., such as Agilent's 2x Hybridization Buffer (part no. 5185-5973), hybridization solutions provided in Agilent's In situ Hybridization Kit Plus (part no. 5184-3568), hybridization solutions provided in Agilent's Deposition Hybridization Kits Plus (part nos. 5184-3526 and 5184-3527), hybridization solutions provided in Agilent's Large-volume Deposition Hybridization Kit (part no. G4145A), and the like.

**Please replace paragraph 121, with the following rewritten paragraph:**

In any event, embodiments include maintaining the substrates of the backing element and the array assembly a certain distance apart such that a seal or barrier is provided around the one or more array of the array assembly formed by the walls of a gasket and the surfaces of the backing element and array substrate. In those embodiments having more than one gasket, the same or different sample may be introduced to the thus provided array assay areas, for example when it is desirable to test the same sample with different arrays during the same assay procedure, or a different sample may be applied to one or more array assay areas than is applied to one or more other array assay areas, for example when it is desirable to test different samples with the same array or different arrays during the same array assay procedure. In any event, a sample may be introduced into an array assay area using any convenient protocol, e.g., by manual or automated means. For example, such may be accomplished using **[[an]]** automated fluid introduction systems employing automated valves and pumps, or other fluid deposition type protocols may be employed, e.g., by manual pipette or the like.

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**Please replace paragraph 129, with the following rewritten paragraph:**

In accordance with the subject methods, one or more of a number of different gaseous components may be removed from the fluid, where the gaseous component(s) may be a pure substance or a mixture and may be one or more of a broad range of chemical species including, but not limited to, gases such as helium, hydrogen, neon, nitrogen, argon, oxygen, ozone, carbon dioxide, ~~ozone~~ and any mixture of any of them. Certain embodiments may include the removal of at least oxygen and/or oxygen mixtures such as oxygen/ozone, oxygen/nitrogen, oxygen/nitrogen/ozone, and the like.

**Please replace paragraph 132, with the following rewritten paragraph:**

Following the washing procedure the one or more arrays may then be interrogated or read so that the presence of any resultant binding complexes on the array substrate surface may be detected, e.g., through use of a signal production system, e.g., an isotopic or fluorescent label present on the analyte, etc. The presence of the analyte in the sample is then deduced from the detection of binding complexes on the array substrate surface.

**Please replace paragraph 138, with the following rewritten paragraph:**

Once the array assay station with the degassing backing element is pre-assembled or pre-packaged therein is received by a user at the second site, an array assembly may be operably positioned on or in the array assay station and an array assembly/degassing backing element structure may be provided by bringing the array assembly and degassing backing element in sufficiently close proximity to each other. A sample may be introduced to the array assay chamber provided by the array assembly/degassing backing element structure, where the order thereof may be reversed or otherwise altered as convenient for a given procedure. The sample may then be incubated with the array(s) of the array assembly, during which time the sample may be degassed periodically or continuously. Following completion of the

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array assay, the substrate having at least one array may be removed from the array assay station, positioned on an array scanner or reader and the at least one array may be scanned by the array reader to obtain a result, as described above.